CLAIMS

1. A method for detecting a mutation indicative of fragile X syndrome, which method comprises the steps of:

(a) obtaining genomic DNA to be tested,

(b) using PCR to amplify nucleic acid along the X-chromosome in the genomic DNA which includes all of the CGG repeats of the untranslated portion of the FRAXA gene plus a substantial contiguous segment of nucleic acid adjacent to said CGG repeats,

(c) obtaining single-stranded product from the amplified nucleic acid of step (b),

(d) hybridizing colorimetric-labeled oligonucleotides which target for (i) (CGG) repeats and (ii) said contiguous nucleic acid segment with said single-stranded product of step (c),

(e) binding said single-stranded product of step (c) to a solid phase,

(f) separating said hybridized product of step (d) from the remainder of the target material,

(g) recovering the labeled target material from the separated product of step (f),

(h) then hybridizing the recovered labeled target material of step (g) to a microarray having a plurality of spots containing suitable oligonucelotide probes complementary thereto,

(i) following hybridization to the microarray, measuring the colorimetric intensities of the hybridized labeled target material present at specific spots on the microarray to obtain individual values therefor, and

(j) comparing the results of step (i) with results from known control samples to accurately quantify the number of CGG repeats in the FRAXA gene of the obtained genomic DNA.

2. The method of claim 1 wherein the number of CGG repeats is determined using the following formula:

$N = 30 + (A - 1.03)66.4$ where N is the number of repeats and A is the ratio of the CI of the target which hybridized with CGG probes to the CI of the target which hybridized to the probes for the contiguous nucleic segment.

3. The method of claim 1 wherein said nucleic acid which is amplified includes the CGG repeat section on the X-chromosome and at least a substantial portion of the 3′ translated segment of the FRAXA gene.

4. The method of claim 1 wherein said the hybridizing target for the CGG repeats contains between 3 and 7 triplets and wherein the CGG repeat probes contain between 6 and 20 triplets and at least twice as many triplets as said repeat target.

5. The method of claim 1 wherein the labeled target carries a fluorescent dye at 5′ end thereof.

6. The method of claim 1 wherein step (b) employs pairs of forward and reverse primers which are complementary to the 3′ borders of a DNA region that includes the entire CGG repeat section and said contiguous segment of the nucleic acid containing at least 30 nucleotides.

7. The method of claim 6 wherein said contiguous segment is 3′ of the CGG repeat section and contains at least a substantial portion of the translated segment of the FRAXA gene.

8. The method of claim 6 wherein the forward primer employed in step (b) which is complementary to the 3′ border of the antisense strand of the DNA region has an anchoring moiety at the 5′ end thereof.

9. The method of claim 8 wherein the reverse primer has phosphate at its 5′ end and said single-stranded product obtained in step (c) is obtained by digesting the antisense strand of the double-stranded PCR product with an exonuclease.

10. The method of claim 9 wherein the anchoring moiety is biotin and wherein steps (e) and (f) are carried out following step (d) and separate said hybridized products by binding to avidin that is attached to a solid phase and washing.

11. The method of claim 10 wherein said labeled target material which hybridized in step (d) is recovered in step (g) by treating said hybridized product of step (d) to denature said strands and collecting the supernatant.

12. The method of claim 9 wherein, following step (b), the amplified nucleic acid product is purified to remove unbound primers prior to treating with the exonuclease to obtain the single-stranded target material.

13. The method of claim 6 wherein said forward primer includes SEQ ID NO: 1 and said reverse primer includes SEQ ID NO: 2.

14. A method for detecting a mutation indicative of fragile X syndrome, which method comprises the steps of:

(a) obtaining genomic DNA to be tested,

(b) using PCR and forward and reverse primers to amplify nucleic acid along the X-chromosome in the genomic DNA which includes all of the CGG repeats and a contiguous portion of the translated FRAXA gene, said forward primers having an anchoring moiety at the 5′ end thereof,

(c) purifying the double-stranded product of step(b),

(d) obtaining single-stranded product from step (c) by digesting the antisense strand thereof with an exonuclease,

(e) hybridizing the product of step (d) with fluorescence-labeled antisense targets for (CGG) repeats and for the contiguous portion of the FRAXA gene,

(f) separating said hybridized product of step (d) from the remainder of nonhybridized targets by binding to a solid phase through said anchoring moieties at the 5′ ends of said forward primers,

(g) hybridizing the product of step (g) to a microarray containing suitable probes and, following hybridization to said microarray, measuring the fluorescent intensities of fluorescence-labeled target material present to obtain individual values therefore, and

(h) comparing the results of step (g) with results from known control samples using the following formula:

$N = 30 + (A - 1.03)66.4$ where N is the number of repeats and A is the ratio of the FI of the target which hybridized with CGG probes to the FI of the target which hybridized to the probes for the contiguous segment, to accurately quantify the number of CGG repeats in the FRAXA gene of the DNA obtained.

15. A method for detecting a short tandem repeat polymorphism (STRP), which method comprises the steps of:

(a) obtaining genomic DNA to be tested,

(b) using PCR to amplify nucleic acid along the chromosome in the genomic DNA which includes all of the STRs of interest plus a substantial contiguous segment of the nucleic acid adjacent to said STRs,

(c) obtaining single-stranded product from the amplified DNA of step (b),

(d) hybridizing colorimetric-labeled oligonucleotides which target for (i) STRs and (ii) said contiguous nucleic acid segment with said single-stranded product of step (c),

(e) binding said single-stranded product of step (c) to a solid phase,

(f) separating said hybridized product of step (d) from the remainder of the labeled target material,

(g) recovering the labeled target material from the product of step (f),

(h) then hybridizing the recovered labeled target material of step (g) to a microarray having a plurality of spots containing suitable oligonucleotide probes complementary thereto,

(i) following hybridization to the microarray, measuring the colorimetric intensities of the hybridized labeled target material present at specific spots on the microarray to obtain individual values therefor, and

(j) comparing the results of step (i) with results from known control samples to accurately quantify the number of STRs in the region of interest of the obtained DNA.

16. The method of claim 15 wherein said the hybridizing target for the STRs contains between 3 and 7 repeats, and wherein the STR probes contain between 6 and 20 repeats and at least twice as many repeats as said STR target.

17. The method of claim 15 wherein step (b) employs a pair of forward and reverse primers which are complementary to the 3′ borders of a DNA region that includes the entire STR section and said contiguous segment of the nucleic acid containing at least 30 nucleotides, the forward primer employed in step (b) being complementary to the 3′ border of the antisense strand of the DNA region and having an anchoring moiety at the 5′ end thereof, and the reverse primer having phosphate at its 5′ end, wherein said labeled target material carry fluorescent dye at 5′ ends thereof, and wherein said single-stranded product is obtained in step (c) by digesting the antisense strand of the double-stranded PCR product with an exonuclease.

18. A kit to detect a mutation indicative of fragile X syndrome, which kit comprises:

(a) a pair of DNA oligonucleotides that will function as forward primers and reverse primers in a polymerase chain reaction (PCR) for amplifying mammalian genomic DNA, wherein the forward primer is complementary to a 3′ nucleotide sequence of the antisense strand of the X-chromosome at a location therealong which is 5′ of the untranslated region of the FRAXA gene and the reverse primer is complementary to a location within the FRAXA gene or 3′ thereof,

said forward primer having an anchoring moiety covalently linked to the 5′ end thereof,

said reverse primer having phosphate at its 5′ end, and

said pair of primer oligonucleotides being specific to amplify the region of genomic DNA which contains all of the CGG repeats and a substantial contiguous segment that serves as an internal control,

(b) labeled oligonucleotides which separately target said CGG repeat region and said internal control segment,

(c) buffers and enzymes for carrying out (i) a PCR, (ii) digestion of the antisense strand, (iii) DNA – DNA hybridizations and washing, (iv) dissociation of hybridized labeled oligonucleotide targets; and (v) colorimetric quantitation,

(d) at least one microarray having a plurality of spots, which spots each have attached thereto DNA probe complementary to one of said labeled oligonucleotide targets; and

(e) means for performing diagnosis for the number of CGG repeats using the results of colorimetric scanning of said microarray and earlier generated data from control samples.

19. The kit of claim 18 wherein one said hybridizing target contains between 3 and 7 CCG repeats, and wherein the CGG probes contain between 6 and 20 repeats and at least twice as many repeats as said complementary target, wherein said forward and reverse primers are complementary to the 3′ borders of a DNA region that includes the entire CGG repeat section and said contiguous segment of the nucleic acid which is 3′ thereof and contains at least 30 nucleotides, the forward primer being complementary to the 3′ border of the antisense strand and having biotin at the 5′ end thereof, and the reverse primer having phosphate at its 5' end, wherein said labeled target oligonucleotides carry fluorescent dye at 5' ends thereof, wherein an amount of STR region target at least about 10 times the amount of the internal control region target is provided, and wherein an exonuclease is provided to digest the antisense strand of the double-stranded PCR product to obtain single-stranded PCR product.

20. A kit to detect a mutation indicative of STRP which kit comprises:

(a) a pair of DNA oligonucleotides that will function as forward primers and reverse primers in a polymerase chain reaction (PCR) for amplifying mammalian genomic DNA, said pair of primer oligonucleotides being specific to amplify a selected region of genomic DNA which contains all of the STRs and a substantial contiguous segment that serves as an internal control; wherein the forward primer is complementary to a 3′ nucleotide sequence of the antisense strand of the selected region of the chromosome and the reverse primer is complementary to the 3′ end of the sense strand of the selected region,

said forward primer having an anchoring moiety covalently linked to the 5′ end thereof, and said reverse primer having its 5′ end blocked to elongation,

(b) labeled oligonucleotides which separately target said STR region and said internal control segment,

(c) buffers and enzymes for carrying out (i) a PCR, (ii) digestion of the antisense strand, (iii) DNA – DNA hybridizations and washing, (iv) dissociation of hybridized labeled oligonucleotide targets; and (v) colorimetric quantitation,

(d) at least one microarray having a plurality of spots, which spots each have attached thereto DNA probe complementary to one of said labeled oligonucleotide targets, and

(e) means for performing diagnosis for the number of STRs using the results of colorimetric scanning of said microarray and earlier generated data from control samples.

21. A kit according to claim 20 which includes solid-phase material having coupling agents which are complementary to said anchoring moieties.